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Sephadex Gel Filtration of γ-Glutamyl-Transpeptidase, Alkaline Phosphatase and Leucine Aminopeptidase in the Serum of Patients Affected by Various Liver Diseases

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γ-Glutamyltranspeptidase, alkaline phosphatase and leucine aminopeptidase activities in the sera of patients affected by various liver diseases were identified after gel filtration on Sephadex G 200.
Protein was scanned at 280 nm and the 19 S, 7 S and 4 S peaks obtained were used as points of reference.
Only one peak of γ-glutamyltranspeptidase activity, at the level of the 19 S protein fraction, was found in the sera of all patients studied.
Alkaline phosphatase and leucine aminopeptidase activity was divided into two peaks corresponding to the 19 and 7 S protein fractions.
In cholestasis cases, whatever the pathology, alkaline phosphatase and leucine aminopeptidase activity present in the 19 S fraction were always more than 20% of the total activity, often reaching values above 40% of the total.

Im Serum von Patienten mit verschiedenen Lebererkrankungen wurden die Aktivitäten der γ-Glutamyltranspeptidase, alkalischen Phosphatase und Leucin-Aminopeptidase nach Gelfiltration an Sephadex G 200 untersucht.
Proteine wurden bei 280 nm gemessen und die 19 S-, 7 S- und 4 S Peaks als Bezugspunkte verwendet.
Im Serum aller untersuchten Patienten wurde nur ein Aktivitäts-Peak der γ-Glutamyltranspeptidase im Bereich der 19 S-Fraktion gefunden.
Die Aktivitäten der alkalischen Phosphatase und Leucin-Aminopeptidase verteilten sich auf zwei Peaks, die den 19 S- und 7 S-Fraktionen zugehörten. In Fällen von Cholostase jeglicher Ätiologie betrugen die Aktivitäten von alkalischer Phosphatase und Leucin-Aminopeptidase in der 19 S-Fraktion stets mehr als 20% und erreichten oft Werte über 40% der Gesamtaktivität.

Although many workers, using different methods, have proved the presence of γ-glutamyltranspeptidase (EC 2.3.2.1), alkaline phosphatase (EC 3.1.3.1) and leucine aminopeptidase (EC 3.4.1.1) isoenzymes in the serum in various disease states, the results are not in agreement and generally are not of sufficient clarity for application to clinical work. This paper reports data concerning the estimation of these isoenzymes by means of gel filtration on Sephadex G 200 in the serum of patients with various liver diseases.

Previous chromatographic estimations of γ-glutamyltranspeptidase by means of gel filtration have been entirely contradictory. Orlowski and Szczeklik (1) found three peaks of enzyme activity: each peak was eluted with one of the three proteic fractions: the highest γ-glutamyltranspeptidase activity was eluted with albumin. Kokot and Kuska (2), Jacyszyn and Laursen (3) also found three peaks: they reported that the highest one was eluted with the 19 S fraction. As far as the other two peaks are concerned, Kokot and Kuska found them in the second protein fraction, while the other workers found them before the first fraction.

Only a few data are available about the gel filtration of alkaline phosphatase activity on Sephadex G 200. In normal serum a fraction of alkaline phosphatase activity at the level of the second peak eluted from the gel has been reported (4): in about 30% of the cases a zone of activity (about 5% of the total activity) has been noted at the level of first peak. In disease, two fractions are always observed: Dunne and coworkers (5) showed that the first fraction is about 2% of the total activity in non-neoplastic bony diseases, 10% in neoplastic bony diseases, 12% in non-neoplastic liver diseases and 32% in metastatic liver tumours. According to these authors, therefore, the high concentration of alkaline phosphatase activity present in the first fraction is characteristic of metastatic tumours. Recently Jennings and coworkers (6) seem to have confirmed these results.

Material and methods

Sephadex gel filtration

We performed gel filtration on Sephadex G 200 (Pharmacia-Upsala, Sweden). Column size was 45 × 2.5 according to Flodin and Killander's method (7). Two ml of serum were applied to the column for each chromatographic estimation. Tris buffer 0.1M pH 8.1 + NaCl 0.5M was used for the elution.
Protein concentration was measured at 280 nm by a Uvicord Automatic Ultraviolet Absorption Detector. The eluate was collected automatically in 3 ml fractions.

Determination of γ-glutamyltranspeptidase

We determined the γ-glutamyltranspeptidase activity by the method of Szasz (8), using L-γ-glutamyl-*p*-nitroanilide as substrate.

Determination of alkaline phosphatase

Alkaline phosphatase activity was determined by the kinetic test at 25° and a wave length of 405 nm, the substrate being *p*-nitrophenylphosphate (9).

As we used a pH 8.1 buffer for gel filtration, it was necessary to correct the pH of the mixture in the cuvette to pH 10.5 with NaOH, because this pH is required for the maximum activity of alkaline phosphatase.

Determination of leucine aminopeptidase

Leucine aminopeptidase activity, was also determined by the kinetic test at 25° and a wave length of 405 nm. This method utilizes as substrate L-leucyl-*p*-nitroanilide (10).

The case material included 8 patients with acute hepatitis, 7 with chronic hepatitis, 8 with portal cirrhosis, 21 with cholestasis (1 with cancer of the head of the pancreas, 1 with cancer of choledochus, 4 with choledocholithiasis, 7 with primary or metastatic liver neoplasms, 1 with cholestasis in HODGKIN disease, 3 with cholangitis, 3 with primary or secondary biliary cirrhosis and 1 with drug cholestasis).

The diagnosis was based upon clinical, biochemical and, in most cases, histological or peritoneoscopic criteria.

In some of these cases, after gel filtration of serum, only one or two enzymes were estimated.

In our laboratories normal values for γ-glutamyltranspeptidase are 8—28 U/l of serum, for alkaline phosphatase 12—30 U/l of serum and for leucine aminopeptidase 8—22 U/l of serum.

Results

Figure 1 shows as a standard the protein profile of normal serum, submitted to gel filtration on a Sephadex G 200 column. The proteins are eluted in three main peaks corresponding to the 19 S, 7 S and 4 S (and albumin) fractions, as defined by ultracentrifugation (7).

γ-Glutamyl transpeptidase activity appears only in the first eluted protein fraction. Alkaline phosphatase and leucine aminopeptidase are present either in the first or in the second eluted protein fraction: but the activity is mainly located in the second fraction, because the first fraction levels never exceed 10% of the total activity.

Tables 1, 2 and 3 show the distribution of γ-glutamyltranspeptidase, alkaline phosphatase and leucine aminopeptidase in the three eluted protein fractions in the morbid forms we considered. It is evident that γ-glutamyltranspeptidase is always present only in the first fraction eluted from the gel, and it shows no difference between any of the liver diseases.

Alkaline phosphatase activity, on the other hand, shows variations: in acute hepatitis, chronic hepatitis and cirrhosis, the activity which appears in the first eluted fraction is quite small — often indeterminate — and

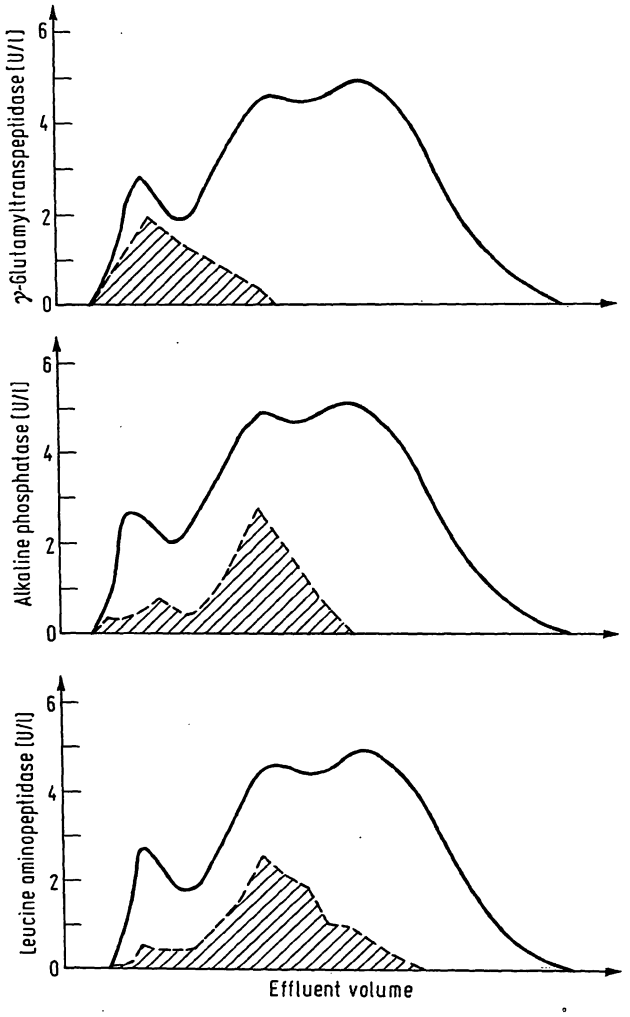


Fig. 1
Gel filtration patterns of sera from normal subjects. Shaded areas: activity of γ-glutamyltranspeptidase, alkaline phosphatase and leucine aminopeptidase

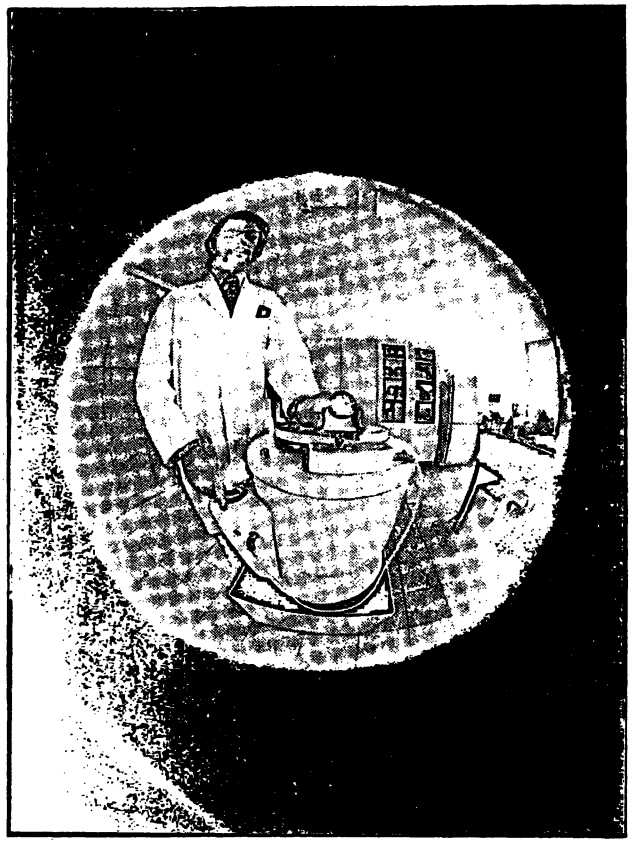
Tab. 1
Gel filtration pattern of γ-glutamyltranspeptidase in serum of patients with various liver diseases. 7S peak and 4S peak were absent

Case No.	Diagnosis	Total activity U/l of serum	% enzyme distribution 19 S peak
1	Infective hepatitis	85	100
2	Infective hepatitis	17	100
3	Infective hepatitis	80	100
4	Infective hepatitis	180	100
5	Chronic hepatitis	27	100
6	Chronic hepatitis	65	100
7	Chronic hepatitis	140	100
8	Portal cirrhosis	300	100
9	Portal cirrhosis	111	100
10	Drug cholestasis	858	100
11	Cholangitis	388	100
12	Cholangitis	410	100
13	Benign obstruction (stone)	220	100
14	Primary biliary cirrhosis	290	100
15	Primary biliary cirrhosis	840	100
16	Secondary biliary cirrhosis	611	100
17	Malignant hepatoma	122	100
18	Malignant hepatoma	155	100
19	Secondary carcinoma liver	420	100
20	Secondary carcinoma liver	275	100
21	Secondary carcinoma liver	450	100

Tabl. 2.
Gel filtration pattern of alkaline phosphatase in serum of patients with various liver diseases. 4 S peak were absent

Case No.	Diagnosis	Total activity U/l of serum	% enzyme distribution 19S peak	7S peak
1	Infective hepatitis	50	6	94
2	Infective hepatitis	82	—	100
3	Infective hepatitis	55	18	82
4	Infective hepatitis	71	—	100
5	Infective hepatitis	40	—	100
6	Infective hepatitis	93	3.5	96.5
7	Chronic hepatitis	22	—	100
8	Chronic hepatitis	93	4	96
9	Chronic hepatitis	66	12	88
10	Portal cirrhosis	44	7.5	92.5
11	Portal cirrhosis	53	—	100
12	Portal cirrhosis	27	—	100
13	Portal cirrhosis	48	5	95
14	Drug cholestasis	104	48	52
15	Cholangitis	188	48	52
16	Benign obstruction (stone)	155	47	53
17	Benign obstruction (stone)	89	22	78
18	Primary biliary cirrhosis	90	42	58
19	Carcinoma ampolla of VATER	64	38	62
20	Carcinoma of head of pancreas	210	42	58
21	Cholestasis in HODGKIN disease	113	40	60
22	Malignant hepatoma	155	20	80
23	Malignant hepatoma	80	37	63
24	Sec. carcinoma liver	85	52	48

Ihm kann „keener"...



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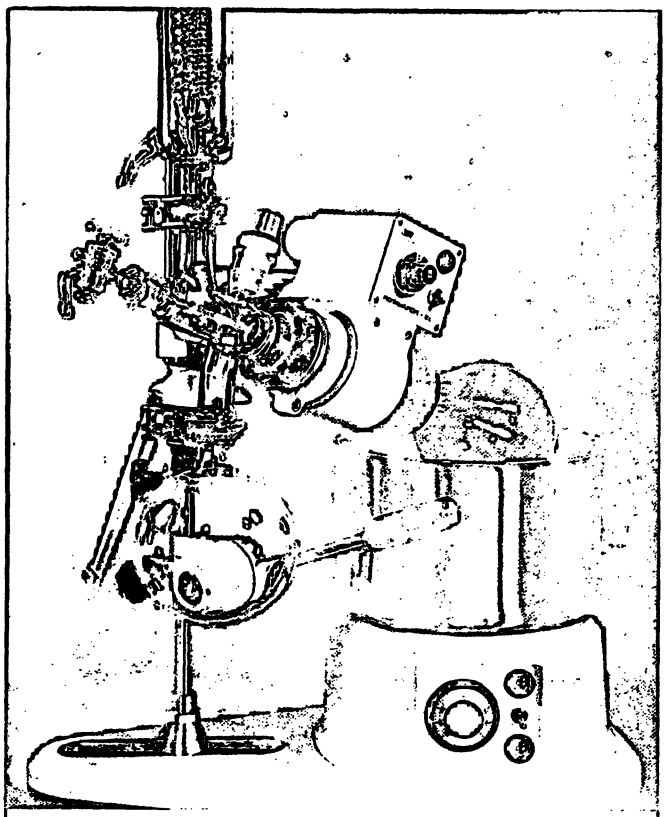

Büchi-
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International bewährter Rotationsverdampfer der bekannten Rotavapor-Baureihe der Firma W. Büchi, Flawil (Schweiz). Elektronisch gesteuerte und stufenlos einstellbare Drehzahl mit konstantem Drehmoment im gesamten Bereich von 10 bis 220 U/min (auch bei voller Belastung). Patentierte Dampfdurchführung ohne bewegte Teile,

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Tab. 3
Gel filtration pattern of leucine aminopeptidase in serum of patients with various liver diseases. 4S peak were absent

Case No.	Diagnosis	Total activity U/l of serum	% enzyme distribution 19S peak	7S peak
1	Infective hepatitis	30	5	95
2	Infective hepatitis	38	12	88
3	Infective hepatitis	45	4	96
4	Chronic hepatitis	24	18	82
5	Chronic hepatitis	55	9	91
6	Chronic hepatitis	18	20	80
7	Chronic hepatitis	16	8	92
8	Chronic hepatitis	36	26	74
9	Portal cirrhosis	24	27	73
10	Portal cirrhosis	46	12	88
11	Portal cirrhosis	48	31	69
12	Portal cirrhosis	52	28	72
13	Portal cirrhosis	35	25	75
14	Portal cirrhosis	31	11	89
15	Drug cholestasis	68	47	53
16	Cholangitis	80	52	48
17	Benign obstruction (stone)	54	52	48
18	Benign obstruction (stone)	110	19	81
19	Primary biliary cirrhosis	46	60	40
20	Carcinoma ampolla of VATER	115	39	61
21	Malignant hepatoma	55	60	40

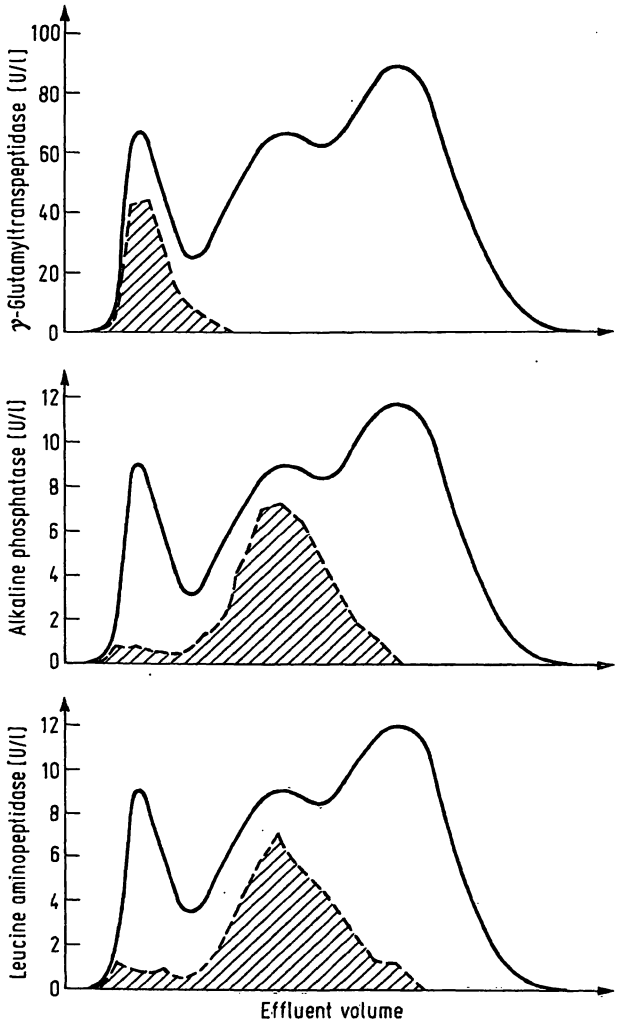

Fig. 2
Gel filtration patterns of sera from patients with acute hepatitis. Shaded areas: activity of γ-glutamyltranspeptidase, alkaline phosphatase and leucine aminopeptidase

always less than 18% of the total activity. In cholestasis, whatever the cause, alkaline phosphatase activity present in the first fraction is always more than 20% of the total, very often reaching values above 40%.

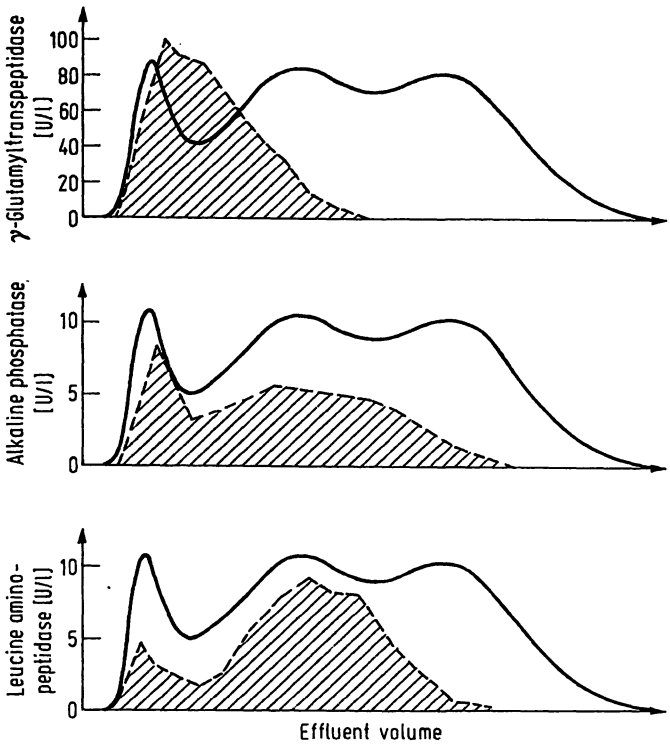

Fig. 3
Gel filtration patterns of sera from patients with cholestatic jaundice. Shaded areas: activity of γ-glutamyltranspeptidase, alkaline phosphatase and leucine aminopeptidase

Leucine aminopeptidase activity appears to behave in a similar fashion. The percentage of leucine aminopepitdase activity present in the first protein peak eluted by gel is quite high in cholestasis. A notable increase of the first fraction was seen even in the cirrhosis cases (values of about 25—30%).

Figures 2 and 3 show as a standard the protein profiles and related zones of activity of γ-glutamyltranspeptidase, alkaline phosphatase and leucine aminopeptidase in patients affected respectively by acute hepatitis and cholestasis.

Discussion

Only one peak of γ-glutamyltranspeptidase activity was found in the sera of all cases studied by gel filtration on Sephadex G 200. This peak was eluted together with the first protein fraction. Therefore γ-glutamyltranspeptidase seems to be a rather homogeneous enzyme with molecular weight about 200,000. However some little differences in molecular weight can be shown when the eluted fractions with γ-glutamyltranspeptidase activities are again characterized by means of gel filtration (11). The different results of other authors might be explained by the formation of multiple molecular forms as speculated by ORLOWSKI and SZCZEKLIK (1) or by the different methods in enzyme assay. It might also be that colorimetric methods used to test γ-glutamyltranspeptidase levels detect non-specific enzyme activities. Otherwise it is difficult to see how LAURSEN and coworkers (3) found γ-glutamyltranspeptidase activity peaks which were eluted even earlier than the heaviest protein fraction.

The detection on Sephadex G 200 of the serial alkaline phosphatase activity showed a characteristic pattern in the sera of patients suffering from cholestasis. In fact only in these patients was it possible to find a high level of alkaline phosphatase activity present in the first eluted fraction. DUNNE and coworkers (5) and JENNINGS and coworkers (6) asserted that this pattern was specific for the serum of patients with primary or metastatic liver tumours: but DUNNE and coworkers have not studied the sera of patients suffering from other forms of cholestasis; and of only two patients affected by benign obstruction considered by JENNINGS and coworkers, one showed high alkaline phosphatase activity (31% of total) in the first fraction. We therefore believe that the presence of a high level at the first peak is found in all cases of cholestasis, whatever the cause.

NEWTON and coworkers (12) and DUNNE and corworkers (5) suggest that this alkaline phosphatase activity related to 19 S fraction could be a lipoprotein complex. The results of JENNINGS and coworkers (6) seem to support this view. These authors observed that, after n-butanol extraction of concentrated 19 S column eluents, alkaline phosphatase activity moved to the 7 S position. On the other hand, an increase in lipoproteins has also been observed in obstructive jaundice (13, 14). However, since in some cases JENNINGS and coworkers observed a considerable loss of alkaline phosphatase activity after n-butanol extraction, we cannot exclude the possibility that alkaline phosphatase activity related to the 19 S fraction may partly be an enzyme polymer (5).

As far as the leucine aminopeptidase activity is concerned, we have observed the presence of two peaks after serum gel filtration, eluting at the level of 19 S and 7 S protein fractions respectively. The changes of the percentages of these two peaks in different liver diseases are similar to the changes in alkaline phosphatase: so the deductions made about alkaline phosphatase activity apply equally to leucine aminopeptidase.

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